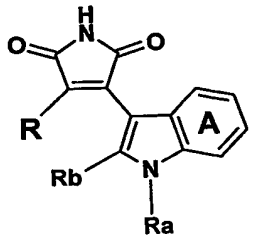
Indolyl-pyrroledione derivatives

The invention relates to the use of compounds (hereinafter: "COMPOUND ") or a N-Oxide or a pharmaceutically acceptable salt thereof having an activity on protein kinases PKC alpha, PKC beta, PKC gamma, PKC epsilon, PKC theta, CDK-1, KDR, PKA, Flt-1, Flt-2, Flt-3 or Flt-4, or on a combination of the above enzymes, for the treatment and/or prevention of neurological and vascular disorders related to beta-amyloid generation and/or aggregation such as neurodegenerative diseases like Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral hemorrhage with amyloidosis.

A compound of formula I



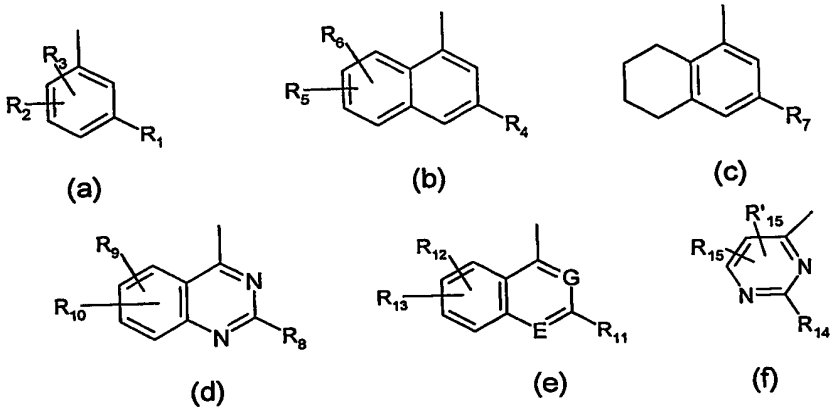
I

wherein

R_a is H; C_{1-4}alkyl; or C_{1-4}alkyl substituted by OH, NH_2, NHC_{1-4}alkyl or $N(di\text{-}C_{1-4}alkyl)_2$;

R_b is H; or C_{1-4}alkyl;

R is a radical of formula (a), (b), (c), (d), (e) or (f)



(a) (b) (c) (d) (e) (f)

wherein

each of R_1, R_4, R_7, R_8, R_{11} and R_{14} is OH; SH; a heterocyclic residue; $NR_{16}R_{17}$ wherein each of R_{16} and R_{17}, independently, is H or C_{1-4}alkyl or R_{16} and R_{17} form together with the nitrogen atom to which they are bound a heterocyclic residue; or a radical of formula α

$$-X-R_c-Y \qquad (\alpha)$$

wherein X is a direct bond, O, S or NR_{18} wherein R_{18} is H or C_{1-4}alkyl,

R_c is C_{1-4}alkylene or C_{1-4}alkylene wherein one CH_2 is replaced by CR_xR_y wherein one of R_x and R_y is H and the other is CH_3, each of R_x and R_y is CH_3 or R_x and R_y form together $-CH_2-CH_2-$, and

Y is bound to the terminal carbon atom and is selected from OH, a heterocyclic residue and $-NR_{19}R_{20}$ wherein each of R_{19} and R_{20} independently is H, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl or C_{1-4}alkyl optionally substituted on the terminal carbon atom by OH, or R_{19} and R_{20} form together with the nitrogen atom to which they are bound a heterocyclic residue;

each of R_2, R_3, R_5, R_6, R_9, R_{10}, R_{12}, R_{13}, R_{15} and R'_{15}, independently, is H, halogen, C_{1-4}alkyl, CF_3, OH, SH, NH_2, C_{1-4}alkoxy, C_{1-4}alkylthio, NHC_{1-4}alkyl, $N(di\text{-}C_{1-4}alkyl)_2$ or CN;

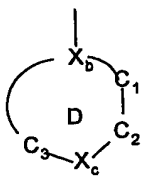
either E is –N= and G is –CH= or E is –CH= and G is –N=; and

ring A is optionally substituted,

or a salt thereof.

Preferably a compound of formula I wherein the heterocyclic residue as R_1, R_4, R_7, R_8, R_{11}, R_{14} or Y or formed, respectively, by $NR_{16}R_{17}$ or $NR_{19}R_{20}$, is a three to eight membered saturated, unsaturated or aromatic heterocyclic ring comprising 1 or 2 heteroatoms, and optionally substituted on one or more ring carbon atoms and/or on a ring nitrogen atom when present.

More preferably a compound of formula I wherein the heterocyclic residue is R_1, R_4, R_7, R_8, R_{11}, R_{14} or Y or formed, respectively, by $NR_{16}R_{17}$ or $NR_{19}R_{20}$, is a residue of formula (γ)



(γ)

wherein

the ring D is a 5, 6 or 7 membered saturated, unsaturated or aromatic ring;

X_b is –N-, -C= or –CH-;

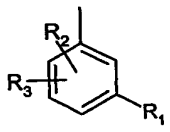
X_c is -N=, -NR_f-, -CR_f'= or –CHR_f'- wherein R_f is a substituent for a ring nitrogen atom and is selected from C_{1-6}alkyl; acyl; C_{3-6}cycloalkyl; C_{3-6}cycloalkyl-C_{1-4}alkyl; phenyl; phenyl-C_{1-4}alkyl; a heterocyclic residue; and a residue of formula β

$$-R_{21}-Y' \qquad (\beta)$$

wherein R_{21} is C_{1-4}alkylene or C_{2-4}alkylene interrupted by O and Y' is OH, NH_2, NH(C_{1-4}alkyl) or N(C_{1-4}alkyl)$_2$; and R_f' is a substituent for a ring carbon atom and is selected from C_{1-4}alkyl; C_{3-6}cycloalkyl optionally further substituted by C_{1-4}alkyl;  wherein p is 1, 2 or 3; CF_3; halogen; OH; NH_2; -CH_2-NH_2; -CH_2-OH; piperidin-1-yl; and pyrrolidinyl;
the bond between C_1 and C_2 is either saturated or unsaturated;
each of C_1 and C_2, independently, is a carbon atom which is optionally substituted by one or two substituents selected among those indicated above for a ring carbon atom; and
the line between C_3 and X_b and between C_1 and X_b, respectively, represents the number of carbon atoms as required to obtain a 5, 6 or 7 membered ring D.
Even more preferably a compound of formula I, wherein D is a piperazinyl ring optionally C- and/or N-substituted as specified in claim 3.

Yet even more preferably COMPOUND a compound of formula I wherein
Ra is H; CH_3; CH_2-CH_3; or isopropyl,
Rb is H; halogen; C_{1-6}alkoxy; or C_{1-6}alkyl, and either

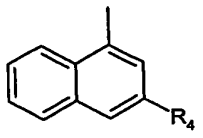
I. R is a radical of formula (a)



(a)

wherein
R_1 is piperazin-1-yl optionally substituted by CH_3 in position 3 or 4; or 4,7-diaza-spiro [2.5] oct-7-yl;
R_2 is Cl; Br; CF_3; or CH_3; and
R_3 is H; CH_3; or CF_3; R_3 being other than H when Ra is H or CH_3, Rb is H and R_1 is 4-methyl-1-piperazinyl; or

II. R is a radical of formula (b)

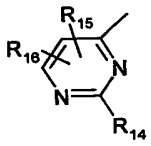


(b)

wherein

R_4 is piperazin-1-yl substituted in positions 3 and/or 4 by CH_3; or 4,7-diaza-spiro [2.5] oct-7-yl; Ra being other than H or CH_3 when R_4 is 4-methyl-1-piperazinyl; or

III. R is a residue of formula (c)



(c)

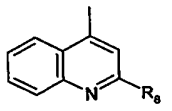
wherein

R_{14} is piperazin-1-yl optionally substituted by CH_3 in position 3 and/or 4 or in position 3 by ethyl, phenyl-C_{1-4}alkyl, C_{1-4}alkoxy-C_{1-4}alkyl or halogeno–C_{1-4}alkyl; or 4,7-diaza-spiro [2.5] oct-7-yl;

R_{15} is halogen; CF_3; or CH_3; R_{15} being other than CH_3 when Ra is H or CH_3, Rb is H and R_{14} is 4-methyl-1-piperazinyl; and

R_{16} is H; CH_3; or CF_3; R_{16} being other than H when R_{15} is Cl, Ra is H or CH_3, Rb is H and R_{14} is 4-methyl-1-piperazinyl; or

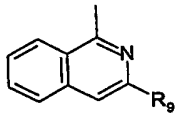
IV. R is a radical of formula (d)



(d)

wherein R_8 is piperazin-1-yl, 3-methyl-piperazin-1-yl or 4-benzyl-piperazin-1-yl; or

V. R is a radical of formula (e)



(e)

wherein R_9 is 4,7-diaza-spiro [2.5] oct-7-yl; or piperazin-1-yl substituted in position 3 by methyl or ethyl and optionally in position 4 by methyl.

The compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example, hydrochloric acid, acetic acid, trifluoroacetic acid. It will be appreciated that the compounds of formula I may exist in the form of optical isomers, racemates or diastereoisomers. For example, a ring carbon atom bearing a substituent in the position 3 of the piperazinyl residue is asymmetric and may have the D- or

L- configuration. It is to be understood that the present invention embraces all enantiomers and their mixtures. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms as mentioned.

An especially preferred COMPOUND is a compound of formula I, as herein before described, wherein

when R is of formula (a)

R_1 is -(4-methyl-piperazin-1-yl), 1-piperazinyl, 3-methyl-piperazin-1-yl or -(4,7-diaza-spiro[2.5]oct-7-yl)

R_2 is 2-Cl or 2-CH_3

R_3 is 3-CH_3, 3-CF_3or H

R_a is H or CH_3

And when,

R is of formula (b)

R_4 is -(4,7-diaza-spiro[2.5]oct-7-yl), 3-methyl-piperazin-1-yl or 4-methyl-3-methyl-piperazin-1-yl

R_a is H orCH_3

And when

R is of formula (c)

R_{14} is -4-methyl-piperazin-1-yl, 3-methyl-piperazin-1-yl, -4,7-diaza-spiro[2.5]oct-7-yl, 1-piperazinyl, 4-methyl-3-methyl-piperazin-yl, 3-methoxyethyl-piperazin-1-yl, 3-ethyl-piperazin-1-yl, 3-benzyl-piperazin-1-yl or 3-CH_2F-piperazin-1-yl

R_{15} is Cl, Br, CF_3, F

R_{16} is CH_3, H, CH_2-CH_3

R_a is H or CH_3

R_b is H, CH_2-CH_2-CH_3, F, $CH(CH_3)_2$, Cl, OCH_3, CH_3 or CH_2-CH_3

And when

R is of formula (d)

R_8 is 3-methyl-piperazin-1-yl, 4-benzyl-1-piperazinyl or 1-piperazinyl

R_a is CH_3 or H

And when

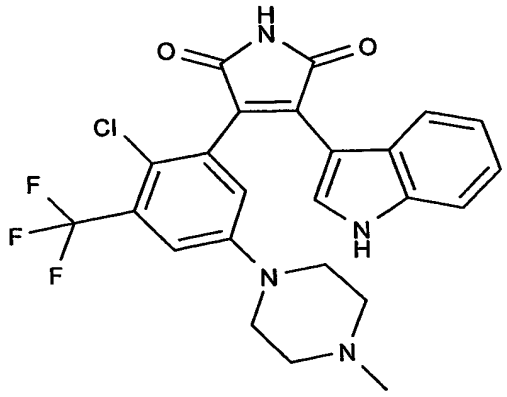
R is of formula (e)

R_9 is -4,7-diaza-spiro[2.5]oct-7-yl, 3-ethyl-piperazin-1-yl, 3-methyl-piperazin-1-yl, 4-methyl-3-methyl-piperazin-1-yl or 3-ethyl-piperazin-1-yl

R_a is H, CH_2-CH_3 or $CH(CH_3)_2$

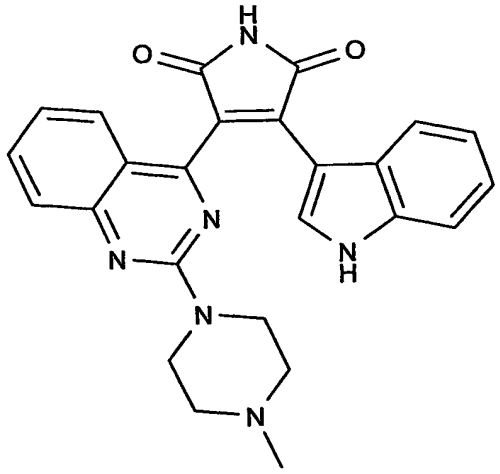
R_b is CH_3, F, $CH(CH_3)_2$, OCH_3, CH_2-CH_3 or Cl

most prefered COMPOUND is 3-[2-Chloro-5-(4-methyl-piperazin-1-yl)-3-trifluoromethyl-phenyl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione having the formula



,or

3-(1H-Indol-3-yl)-4-[2-(4-methyl-piperazin-1-yl)-quinazolin-4-yl]-pyrrole-2,5-dione having the formula



Even more preferred, Compound means any of the other definitions of COMPOUND wherein the compound has an activity on PKC alpha, PKC beta, PKC gamma, PKC epsilon, PKC theta, or on a combination of these enzymes.

Compounds of formula I and methods for the preparation of such compounds are in particular generically and specifically disclosed in the patents and patent application WO2003082859, in particular in the compound claims and the final products of the working examples, the subject-matter of the final products, the pharmaceutical preparations and the claims is hereby incorporated into the present application by reference to this publication.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated:

Alkyl or alkoxy may be straight or branched. Phenyl-C_{1-4}alkyl is preferably benzyl or phenethyl. In C_{1-4}alkoxy-C_{1-4}alkyl the alkoxy moiety is preferably methoxy or ethoxy and the alkyl moiety preferably methyl or ethyl; a suitable example is e.g. 2-methoxyethyl. Halogen may be F, Cl, Br or I, preferably F, Cl or Br. Halogeno–C_{1-4}alkyl is alkyl wherein one or more H are replaced by halogen, e.g. Cl or F, e.g. CH_2Cl, CH_2F or CF_3

R is preferably a radical of formula (a), (c) or (e).

In the radical of formula (a) or (c), R_2 or R_{15} is preferably in para to R_1 or R_{14}, respectively. R_3 is preferably in meta to R_1. In the radical or formula (e), R_9 is preferably 4,7-diaza-spiro [2.5] oct-7-yl.

PKC is protein kinase C

CDK is cyclin dependent kinase

PKA is protein kinase A

Salts are especially the pharmaceutically acceptable salts of compounds of formula I.

Such salts are formed, for example, as acid addition salts, preferably with organic or inorganic acids, from compounds of formula I with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, halogen acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid. Suitable organic acids are, for example, carboxylic, phosphonic, sulfonic or sulfamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid,

fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, amino acids, such as glutamic acid or aspartic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, adamantanecarboxylic acid, benzoic acid, salicylic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, mandelic acid, cinnamic acid, methane- or ethane-sulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 1,5-naphthalene-disulfonic acid, 2-, 3- or 4-methylbenzenesulfonic acid, methylsulfuric acid, ethylsulfuric acid, dodecylsulfuric acid, N-cyclohexylsulfamic acid, N-methyl-, N-ethyl- or N-propyl-sulfamic acid, or other organic protonic acids, such as ascorbic acid.

In the presence of negatively charged radicals, such as carboxy or sulfo, salts may also be formed with bases, e.g. metal or ammonium salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, for example triethylamine or tri(2-hydroxyethyl)amine, or heterocyclic bases, for example N-ethyl-piperidine or N,N'-dimethylpiperazine.

The invention further relates to the use of COMPOUND or a N-Oxide or a pharmaceutically acceptable salt thereof for the manufacture of medicament having an activity on protein kinases PKC alpha, PKC beta, PKC gamma, PKC epsilon, PKC theta, CDK-1, KDR, PKA, Flt-1, Flt-2, Flt-3 or Flt-4, or on a combination of the above enzymes, for the treatment and/or prevention of neurological and vascular disorders related to beta-amyloid generation and/or aggregation such as neurodegenerative diseases like Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral hemorrhage with amyloidosis.

Depending on species, age, individual condition, mode of administration, and the clinical picture in question, effective doses, for example daily doses of about 10-1000 mg, preferably 10-50 mg or 50-200mg or 200-400mg, especially 50-100mg or 300-400 mg, are administered to warm-blooded animals of about 70 kg bodyweight. For adult patients with neurological and vascular disorders related to beta-amyloid generation and/or aggregation, especially neurodegenerative diseases like Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral hemorrhage with amyloidosis.

The invention relates likewise to a process or a method for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation, especially neurodegenerative diseases like Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral hemorrhage with amyloidosis. The COMPOUNDS thereof can be administered as such or especially in the form of pharmaceutical compositions, prophylactically or therapeutically, preferably in an amount effective against the said diseases, to a warm-blooded animal, for example a human, requiring such treatment. In the case of an individual having a bodyweight of about 70 kg the daily dose administered is from approximately 0.01 g to approximately 5 g, preferably from approximately 0.25 g to approximately 1.5 g, more preferably 0.01g to 0.05g, even more preferably 0.025g to 0.1g most preferably 0.05g to 1g of a compound of the present invention.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye. The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

The invention relates also to a method for administering to a human subject suffering from a neurological and vascular disorders related to beta-amyloid generation and/or aggregation, especially neurodegenerative diseases like Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral hemorrhage with amyloidosis, COMPOUND or a pharmaceutically acceptable salt thereof, which comprises administering a pharmaceutically effective amount of COMPOUND or a pharmaceutically acceptable salt thereof to the human subject, preferably once daily for a period exceeding 3 months. The invention relates

especially to such method wherein a daily dose of 200 to 800 mg, or 10mg to 200mg especially 400-600 mg or 10–100mg, preferably 400 mg or 10-50mg, of COMPOUND is administered.

The invention also relates in a combination which comprises (a) COMPOUND or a pharmaceutically acceptable salt thereof and (b) a therapeutic agent for the treatment of neurological and vascular disorders related to beta-amyloid generation and/or aggregation, most preferably a combination wherein the combination partners are present in synergistically effective amounts.

The effective dosage of each of the combination partners employed in the combination may vary depending on a variety of factors including the particular combination of the pharmaceutical compound partners, the route of administration, the severity of the disease, the renal and hepatic functions of the patient. The molar ratio (a)/(b) of the combination partners is about 0.1 to 10, most preferably 0.3 to 3 and the unit dosage form contains 20 to 200 mg, most preferably 50 to 150 mg of 3-[2-Chloro-5-(4-methyl-piperazin-1-yl)-3-trifluoromethyl-phenyl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione of the formula I.

Example 1:

Cell culture

HEK/APPswe cells are plated in microtiter plates precoated with 10 µg/ml poly-D-lysine at 12'000 cells/well in 100 µl/well DMEM medium supplemented with 10% FCS, 0.25 mg/ml G418 sulfate, 1% penicillin streptomycin. The following day, supernatant is replaced with 90 µl/well of fresh medium and 10 µl/well of compound diluted in culture medium are added. Two types of control wells are used: cell culture medium without cells plus 10 µl/well of all compound dilutions (background signals) and cell culture medium from untreated cells (positive control). 24 hours later after compound addition, conditioned medium is collected and Aβ levels determined by a specific sandwich ELISA.

$A\beta_{40}$ and $A\beta_{42}$ detection by sandwich ELISA

For the sandwich ELISA, the maxisorp microtiterplates are coated overnight at 4°C with 100 µl/well of the monoclonal antibody 25H10 diluted 1:1000 in PB for $A\beta_{40}$ detection or monoclonal antibody B10E7 diluted 1:2750 for detection of $A\beta_{42}$. Wells are then emptied, washed three times with 350 µl PBS and blocking is performed for 2 hours at room

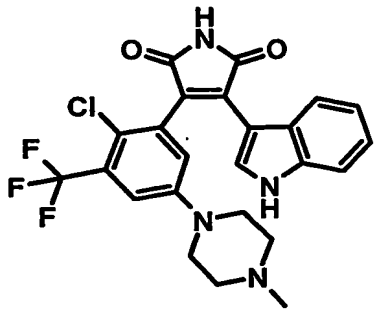
temperature with 200 µl/well of 2 % BSA, 0.05% Tween20 in PBS. After washing the wells as described above, 10 µl of the conditioned media samples to be tested are added to wells containing 90 µl of medium and 0.18 µg/ml of biotinylated monoclonal β1 antibody and incubated overnight at 4°C. Wells were washed as described above and 100 µl/well of alkaline phosphatase coupled to streptavidin diluted 1:5'000 in medium are added. After 1 hour incubation at room temperature wells are washed as described above and alkaline phosphatase activity is determined by adding 100 µl/well of diethanolamine buffer, pH 9.8 (100 mM diethanolamine, 1 mM $MgCl_2$, pH adjusted to 9.8 with 2 M HCl) containing the chemiluminescent CSPD substrate (25 mM stock solution diluted 1:416) and the enhancer Emerald II (diluted 1:10). After 15 minutes incubation at room temperature in the dark, plates are measured on the luminometer (Analyst AD; LJL Biosystems, USA $A\beta_{40}$). Values are given as % reduction of Aβ. The 100% reduction value is calculated from a series of wells containing only medium and extract and the 0% reduction value from conditioned medium only. Samples are measured in triplicate. A reference compound is included in all plates as control for assay performance.

MTS assay

To determine cytotoxicity, cells are tested by the MTS colorimetric kit performed essentially according to the manufacturer's specifications (Promega, #G5430X). After collecting the conditioned medium for the sandwich ELISA, the rest of the conditioned medium is removed completely and replaced with 100 µl/well culture medium containing one fifth of MTS solution prepared as recommended in the kit. After 3 hours incubation at 37°C, absorbance is read at an OD of 490 nm with a reference wavelength set to 630 nm. Values are given as % metabolic rate (n=6). The 0% value is calculated from wells which had no cells, 100% from wells with an untreated cell layer

Example 2:

3-[2-Chloro-5-(4-methyl-piperazin-1-yl)-3-trifluoromethyl-phenyl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione



This compound has the following activities in cell-free enzyme assays:

PKC alpha	21 nM
PKC beta	30 nM
PKC gamma	< 500 nM
PKC epsilon	514 nM
PKC theta	186 nM
CDK-1	< 10 microM
KDR	< 10 microM
PKA	< 10 microM
Flt-1	< 10 microM
Flt-2	< 10 microM
Flt-3	< 10 microM
Flt-4	< 10 microM

The compound of Example 2 demonstrates a clear reduction of Aβ secretion in the medium of HEK/APPswe cell cultures at concentrations below 1 microM, without having any effect on cellular viability.